U.S. EPA BASE STUDY
STANDARD OPERATING PROCEDURE FOR SAMPLING AND CHARACTERIZATION OF BIOAEROSOLS IN INDOOR AIR

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1.0 OBJECTIVE

The objective of the procedure described is to determine concentrations of viable airborne microbiological organisms (bioaerosols) that may be present in indoor air and in the outdoor air supplied to the space tested. The collected samples are subsequently cultured to speciate and quantify the organisms collected. The colony forming units (CFUs) of each organism are quantitated in terms of their number per unit volume (STP) of air sampled. The organisms of interest are mesophyllic bacteria, thermophyllic bacteria and fungi.

Bioaerosols can be collected by gravitational settling, centrifugal force, electrostatic impacting, filtration, or inertial impacting. This latter technique is used under the BASE Protocol. Bioaerosol samples are collected indoors (at Fixed Sites 1, 3, and 5, as defined in the BASE Protocol) and near outdoor air intake for the study area, employing single stage (N6) Anderson inertial impactors with isokinetic sampling probes. Samples are collected on two different types of culture plates. Tryptic soy agar (TSA) culture plates are employed for thermophyllic and mesophyllic bacteria, and malt extract agar (MEA) culture plates for fungi.

An essential criterion for proper collection of a representative sample of aerosol (*i.e.,* sampling all size fractions with equal efficiency) is that the sampling be done under isokinetic conditions. The isokinetic condition is met if the gas velocity at the inlet to the sampling probe is identical to that of the free stream velocity approaching the inlet. The wide sampling port of the Anderson impactor meets the isokinetic criterion. Air is sampled at a flow rate of 28.3±1.4 liters per minute, for time intervals of 2 minutes and 5 minutes as is specified in the BASE Protocol.

The impactor stage selected for sample collection defines the size (aerodynamic diameter) of the particles collected. A single (N6) stage Anderson collector gathers particles with an aerodynamic diameter of 0.65 microns with 50% efficiency.

2.0 GENERAL PROCEDURES

2.1 GENERAL SAMPLING CRITERIA AND REQUIREMENTS

Sampler (Anderson N6) Disinfection. Before each round of sampling the sampler (inlet and impaction plate) is wiped with isopropyl alcohol-soaked cotton swabs. This operation must be conducted in a location away from the BASE study space as integrated sampling of volatile organic compounds (VOCs) is conducted in coincidence with bioaerosol sampling.

Collection Media. The samples are collected on culture media deposited inside covered Petri dishes.[1] These sampling dishes must be sufficiently clean to require no in-field disinfecting. The media is used fresh, *i.e.*, before the expiration date given by the supplier (usually 30 days after receipt). Only media from the same lot is used in any particular building study. A certification of test results under controlled inoculation (Certificate of Analysis) for each lot of culture media dishes must be requested from the supplier. The condition of the culture media must be evaluated for even distribution and coverage of the plates as well as for the presence of growth on the plates. This must be performed upon receipt of the plates to allow time for an appropriate resupply prior to the sampling day.

Concentration Range. To reduce the probability of losing samples because of oversampling or undersampling (too many colony forming units invalidate the count, too few are statistically insignificant), each site is tested twice, at different sample durations. The BASE Protocol, specifies sampling times of 2 minutes (56 liter sample) and 5 minutes (140 liter sample).

[1] Supplied by Regional Media Laboratory, (REMEL), Lawrence, Kansas

2.2 REQUIRED EQUIPMENT AND SUPPLIES

The equipment and supplies required to perform bioaerosol sampling as part of the BASE study is as follows.

- 20 MEA culture plates contained in disposable Petri dishes
- 40 TSA culture plates contained in disposable Petri dishes
- 6 Anderson N-6 single stage samplers
- 6 Sound-insulated pumps with a minimum capacity of 30 lpm (each pump must also be equipped with an appropriate flow control device, such as a needle valve)
- 6 Rotameters calibrated in the range of 26 to 30 lpm
- 1/4" flexible (*e.g.* latex) tubing
- Stopwatch (or automatic, multi set point timer/switch)
- Isopropyl alcohol, cotton balls
- IADCS Sample ID labels (affixed to Petri dish prior to sampling)

2.3 SAMPLING APPARATUS

A sampling cart is used for conducting the bioaerosol sampling as specified in the BASE Protocol. Apparatus used for bioaerosol sampling is attached to the cart, making it convenient to easily move the cart from site to site.

The apparatus consists of six Anderson impactors each connected to a flow meter and a pump of the required flow capacity (28.3±1.4 l/min). The impactors are applied to the top of the sampling cart and the rotameters are attached in a row on the side of the cart. The six sampling pumps are located on the bottom of the sampling cart. To reduce noise generated by the pumps, sound-proofing material covers all four sides of the lower section of the cart. An automatic, programmable timer is used for operating the pumps sequentially for samples of two and five minutes. A schematic diagram of the sampling cart and apparatus is shown in Figure 1 of Appendix A.

2.4 SET-UP AND SAMPLING

All MEA and TSA plates are labeled the night before sampling using the IADCS-generated sample labels. The labels are affixed to the side of the Petri dish, so that they are easily read without rotating the dish. For proper location of the IADCS ID label, refer to Figure 2 of Appendix A. Before use, all plates will be stored in the upside down position to prevent condensation on the media. After sampling, plates are stored and shipped in the right side up position.

Shortly prior to sampling both indoors and outdoors, the internal surfaces of the impactors are wiped with isopropyl alcohol-soaked cotton swabs. This cleaning is done at a location sufficiently removed from the BASE study space fixed sampling sites since integrated sampling of VOCs is being collected in coincidence with bioaerosol sampling. Isopropyl alcohol will cause contamination of the VOC sample.

The samples and their duplicates (at the outdoor site and indoor duplicate site) are collected simultaneously by setting up six impactor plates (2 MEA and 4 TSA) at a time. This is done by uncovering the Petri dish, culture medium side up, and placing it on the bottom plate of the corresponding sampler. Particular care must be taken to insure that each Petri dish is placed evenly on the impactor support pegs and that hands are kept from over the plate so as to avoid contamination deposited by the operator. The impactor plate and top section are then replaced and secured. This procedure is performed for two-minute sampling and again repeated for five minute sampling.

For samples without duplicates, the same six plates, as described above, are set up, however, three of the plates (2 MEA and 1 TSA) are exposed to the air stream for two minutes, while the other three plates collect sample for an additional three minutes. This procedure is easily facilitated by the use of an automatic timer that stops power to three pumps after two minutes and the remaining three pumps after five minutes. The automatic timer should be set to the desired run-time and should then be switched on for sampling. The pump and the stopwatch (or timer) will be turned on simultaneously.

The air flowrate registered by the flow meters connected to each pump and sampler are recorded during the sampling time interval.

After completion of sampling, the impactors are disassembled, the Petri dishes removed and their covers replaced. The samples are shipped to the analyzing laboratory on the same day of sampling.

2.5 SAMPLING SITE LOCATIONS

Samples are collected during the morning and during the afternoon of the day specified in the BASE Protocol at the following locations, provided the test space can accommodate this configuration.

Outdoor Site (near the outdoor air intake for the study area). Two and five minute samples and duplicates for mesophyllic and thermophyllic bacteria and fungi.

Fixed Site 1 (indoors). Two and five minute samples for mesophyllic and thermophyllic bacteria and fungi.

Fixed Site 3 (indoors). Two and five minute samples for mesophyllic and thermophyllic bacteria, fungi, three field blanks collected in the morning round, and three shipping blanks collected in the afternoon round[2].

Fixed Site 5 (indoors). Two and five minute samples and duplicates[3] for mesophyllic and thermophyllic bacteria and fungi.

[2] Field blanks and shipping blanks may be collected at either site F1, F3, or F5.

[3] Indoor duplicate samples may be collected at either site F1, F3, or F5 and may be placed based on site physical restrictions. Indoor duplicate samples shall not be collected across multiple fixed indoor sites (e.g., VOC duplicates at F1, particles at F3, and other duplicate samplers at F5).

3.0 CALIBRATIONS AND QUALITY CONTROL

3.1 QUALITY CONTROL OF CULTURE MEDIA

Each lot of culture medium will be checked for sterility and for ability to support the growth of the organisms most likely to be collected during sampling. The ability to support the organisms to be tested must be certified by the supplier of the culture media.

3.2 QUALITY CONTROL SAMPLES

QC samples consist of Field Blanks and Shipping Blanks. The precision of the measurement is assessed by duplicate sampling.

The possible contamination resulting from handling of the sample media is assessed by analyzing a field blank. The field blank is prepared by going through the process of placing plates in the impactors, removing them (without doing air sampling) and returning them to their Petri dishes. Field blank samples (one each) for mesophyllic and thermophyllic bacteria, and fungi are prepared in the morning round of sampling.

The sterility of the plates is checked by returning one unexposed shipping blank of each medium (TSA and MEA) for each building sampled under the BASE program. The shipping blank is prepared by taking an unused plate (without opening the Petri dish) and submitting it to the laboratory with the other samples. Shipping blanks are performed during the afternoon sampling round.

The repeatability (precision) of sampling and analysis is assessed by sample duplicates. One set of duplicate samples is collected at a specified indoor location and another set of duplicate samples is collected at the outdoor location.

3.3 QUALITY CONTROL OF FLOW RATES

The BASE Protocol specifies a sampling (*i.e.*, air flow) rate of 1 cfm (~28 lpm, ±5%). Prior to and following each seasonal BASE study, the flowrate must be adjusted to this value by turning on the pumps and measuring the flowrate at the inlet to the Anderson impactor with a collection plate in place. This is performed by connecting a calibrated rotameter at the inlet of the impactor and adjusting the control valves on the pump inlet to yield the appropriate flowrate. The inline rotameters placed between each impactor and its pump are then marked for reference to this flowrate measured under atmospheric pressure conditions. An illustration of the sampling train is detailed in Figure 3 of Appendix A.

4.0 SHIPPING AND STORAGE PROCEDURES

The culture plates must not be refrigerated, neither before nor after sampling. In the field and during shipping, the plates are kept inside sealed, well insulated containers to reduce the risk of contamination.

The night before sampling each plate is tagged with an IADCS ID label pasted on the outside edge of the culture medium-supporting base.

The samples are shipped to the analyzing laboratory immediately after sampling for receipt at the laboratory within 24 hours of collection. Arrangements must be made prior to each sample collection sequence to insure timely reception and processing of the samples at the laboratory.

After sampling, all plates will be stored and shipped in the upright position to prevent dislodging of impacted organisms from the plates.

5.0 SAMPLE ANALYSIS

The collected samples must be analyzed, according to BASE Protocol, under the supervision of a person with "demonstrated experience in the handling and analysis of environmental isolates".

The data will be reported as follows:

- cfu/m^3 by genus and cfu/m^3 total for fungi
- cfu/m^3 for gram negative, cfu/m^3 for gram positive, and cfu/m^3 total, for bacteria

APPENDIX A

FIGURES

Figure 1 **Bioaerosol Sampling Cart**

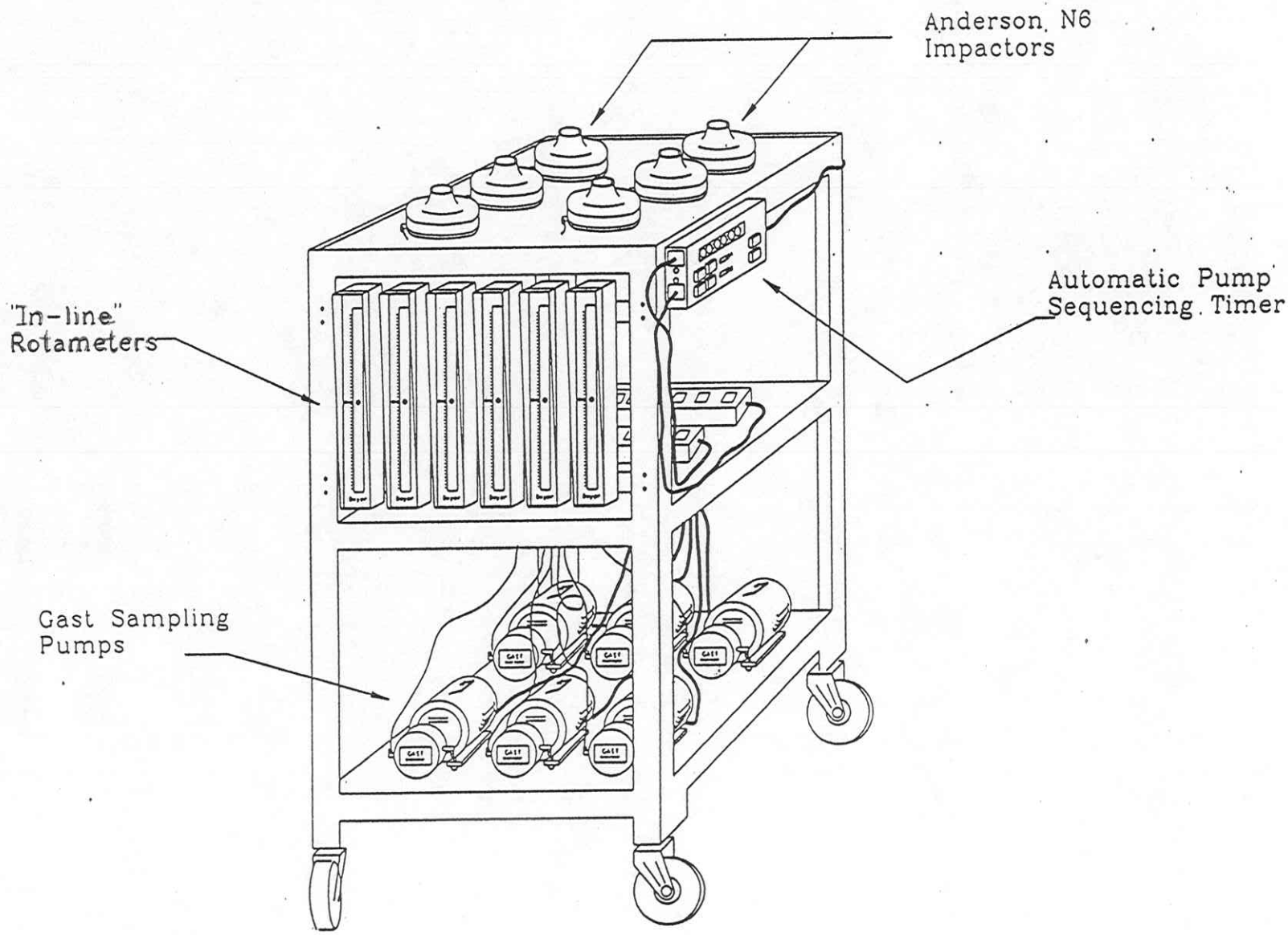
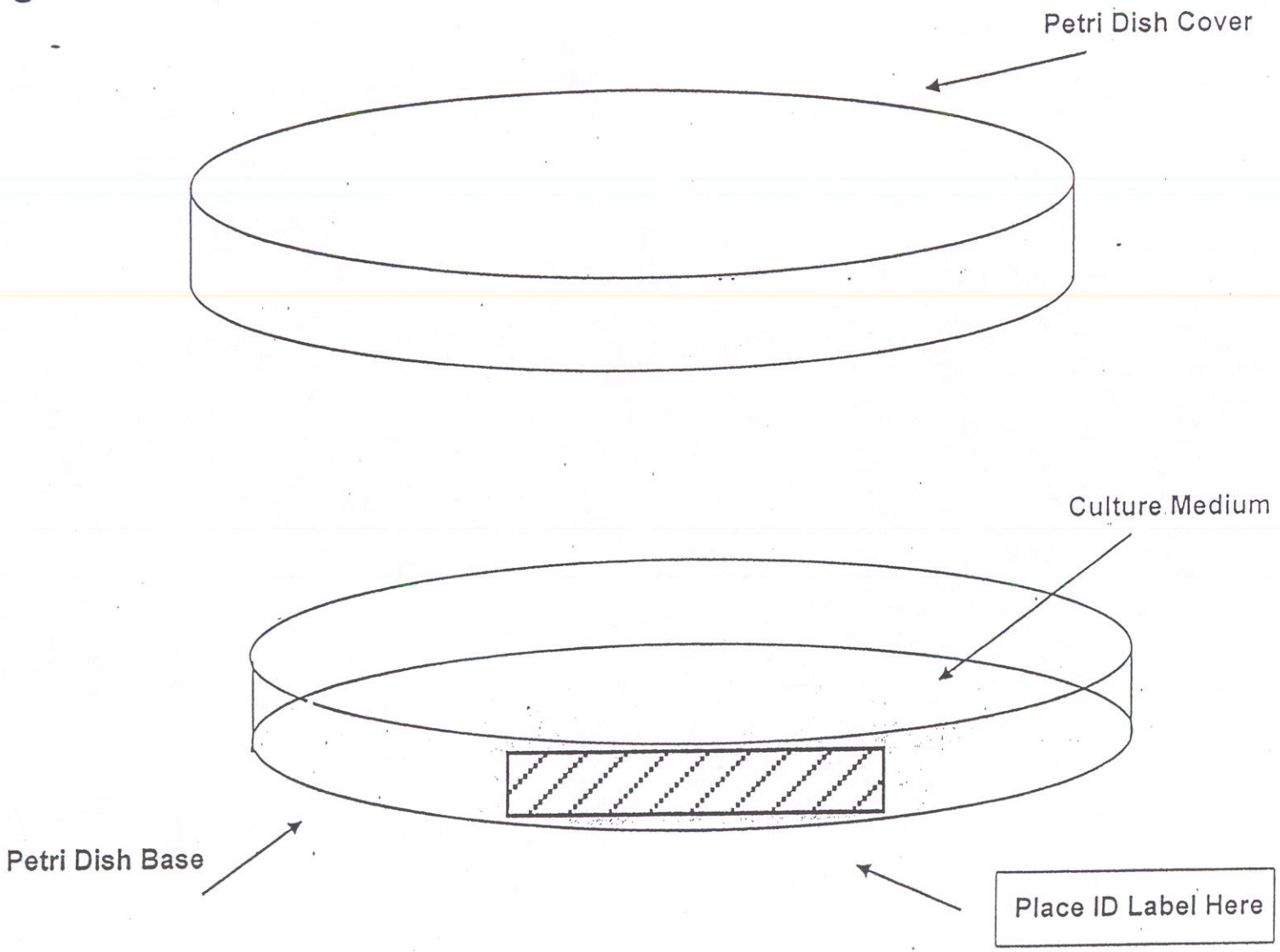


Figure 2


Petri Dish Cover
Culture Medium
Petri Dish Base
Place ID Label Here

28.3 LPM
Dwyer 10-100 Rotas
Matheson Cheapie II
2 + 5 Min Samples
MEA, TSA, Therm TSA

Figure 3